Direct systemic effects of BMP 2 and 7 on the skeleton

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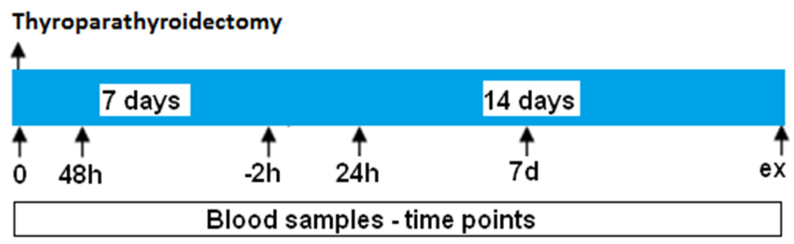
INTRODUCTION

Bone morphogenetic proteins (BMPs) play an important role in the bone and joint regeneration [1-4]. BMP2 and 7 with the bovine collagen carrier were approved for the treatment of spinal fusions, long bone non-unions and acute fractures after randomized, controlled and blinded clinical trials [5,6]. The osteoinductive ability of BMP2 and 7 distinguishes them from other available bone grafts and eliminates the donor-site morbidity in contrast to autologous bone grafting. Moreover, no adverse events were observed in more than 700 patients from randomized clinical trials (RCTs). Recently, both BMP2 based Infuse and BMP7 based Osigraft were confronted with side-effects and unresolved clinical issues [7,8]. Specifically, vertebral implant migration and subsidence as a result of early osteolysis, heterotopic ossification and retrograde ejaculation were among registered complications [7,8]. Additionally, it is unknown whether their systemic release following a local implantation might impact the bone metabolism. Furthermore, it is unknown whether systemic BMP effects on bone are direct or mediated by calciotropic hormones. To answer these questions we examined effects of systemically administered BMP2 and 7 on bone in a newly developed rat model with a low level of calciotropic hormones.

MATHERIALS AND METHODS

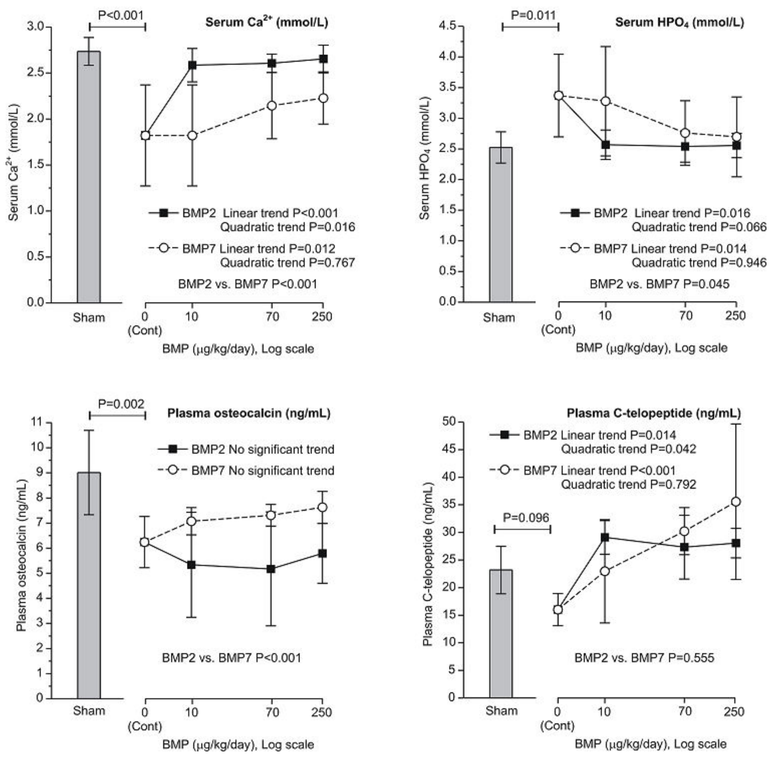
Animals: Removal of thyroid and parathyroid glands was done by a ventral approach under the influence of rompun (Xylapan 0,6 ml/kg i.p.) and ketamine (Narketan 0,8 ml/kg i.p.) anesthesia.

Biochemical serum parameters: blood was collected at various time points by retroorbital bleed for serum chemistry and for determination of bone turnover biomarkers as well as determination of thyroid and parathyroid hormones and vitamin D.



MicroCT: The uCT 1076 and analyzing software were procured from SkyScan. The distal femur and proximal tibia were scanned in 250 slices, each 18um thick in the dorsoventral direction. 3D reconstruction of the bone was performed using the triangulation algorithm. The trabecular bone parameters, such as trabecular bone volume were directly measured on 3D images.

Cell culture: Bone marrow cells were harvested from femurs and tibias of 3 months old rats previously subjected to TPTx, pooled and plated on 24-well plates at density of 1x106 cells per well. Differentiation media for osteoclasts contained α-MEM, 10% FBS, macrophage colony-stimulating factor (M-CSF, 20 ng/mL; Sigma Aldrich), and recombinant human soluble receptor activator of nuclear factor-κB ligand (RANKL, 20 ng/mL; Sigma Aldrich). BMP2 (100 ng/mL) and BMP7 (100 ng/mL) were added to the medium on day 1 and replaced at every feeding until termination on day 6. The cells were fixed with 4% paraformaldehyde, and adherent osteoclasts were identified by tartrate-resistant acid phosphatase (TRAP) staining using a commercially available kit (Sigma Aldrich). Only osteoclasts with three or more nuclei were counted. For RNA isolation and qRT-PCR analysis cells were collected in TRI Reagent (Life Technologies). Differentiation medium for osteoblasts was added on day 7 and contained α-MEM, 10% FBS, 10mM β-glycerophosphate and 50 µg/mL ascorbic acid. The medium was changed every two days until the culture was terminated on day 19. BMP2 (100 ng/mL) or BMP7 (100 ng/mL) were added to the medium at every feeding. Osteoblasts were identified by alkaline phosphatase stain using a commercially available kit (Sigma Aldrich). Von Kossa staining was used to determine the mineralized matrix formation. Quantification of the mineralized area was performed using S-form software and expressed as percentage of the mineralized area.

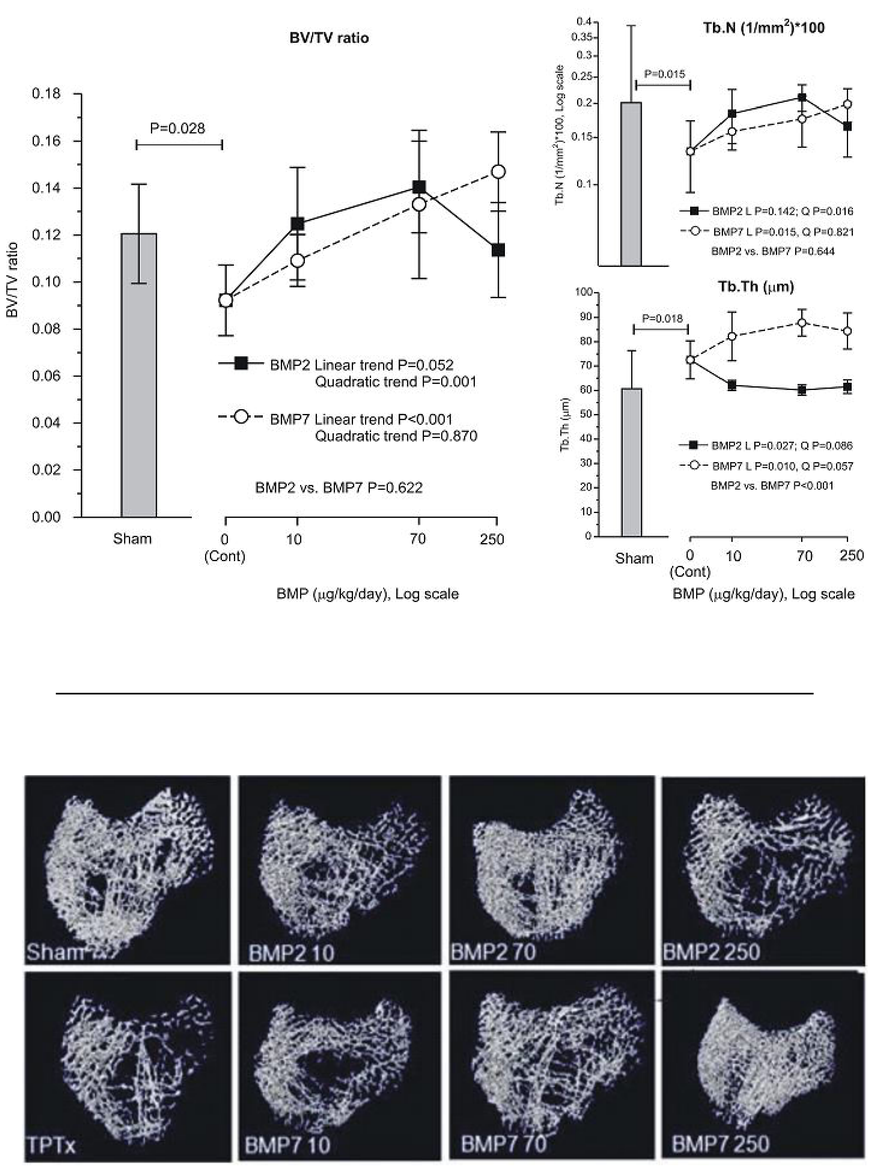


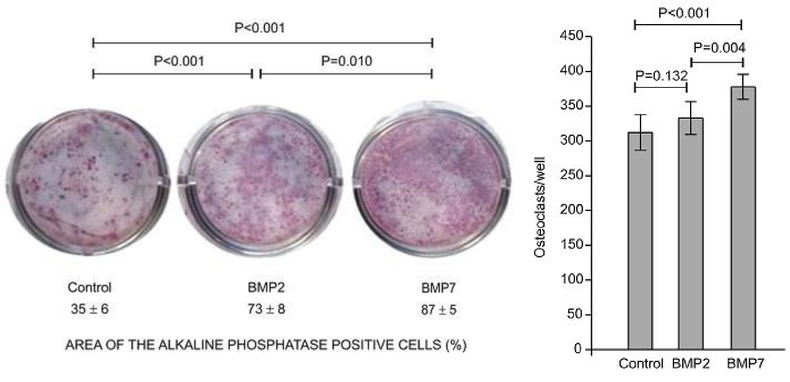
RESULTS

Both BMP2 and 7 dose-dependently increased serum calcium level, but the effect of BMP2 was significantly pronounced. As expected, the phosphate level was in a good correlation with calcium, maintaining the calcium-phosphate product with a similar trend between BMP2 and 7 doses. Osteocalcin and C-telopeptide were both suppressed in TPTx animals, confirming a low remodeling rate. The osteocalcin plasma level in TPTx rats was significantly lower than in sham animals. Neither BMP2 nor BMP7 therapy had a significant effect on osteocalcin plasma level. However, there was an overall significant difference between BMP2 and 7 across all doses in favor of BMP7. On the contrary, plasma C-telopeptide level was lower in TPTx rats with significant positive dose-dependent effects of both BMPs. There was no overall difference between BMP2 and 7 therapy.

A significant bone loss occurred within 7 days after TPTx. Following 14 days of BMP2 and 7 administration µCT of the distal femur was performed to determine their capacity to compensate for TPTx caused bone loss. Both BMP2 and 7 dose-dependently significantly increased the BV/TV in TPTx rats.

Consistent with *in vivo* results, both BMP2 and 7 enhanced the differentiation of bone marrow derived mesenchymal stem cells towards an osteoblastic phenotype as evidenced by increased alkaline phosphatase positive cells and also increased osteoclast number. This suggested that BMP2 and 7 significantly promote both bone formation and resorption in cultured TPTx bone cells.





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